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Hepatitis C Virus Infection in Acquired Aplastic Anemia

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Hepatitis-associated aplastic anemia (HAAA) is an uncommon disorder that usually is not due to hepatitis A or B virus infection. Hepatitis C virus (HCV) seropositivity is infrequently observed in aplastic anemia (AA) patients who have not been extensively transfused. However, HCV seropositivity may not be detected until several weeks or months after viral infection and AA patients may exhibit defective humoral immunity. Therefore, we evaluated sera from AA patients for the presence of HCV viremia using a reverse transcriptase polymerase chain reaction (RT-PCR) based assay and several serologic assays for HCV antibodies. Serum samples from 90 AA patients who presented to the UCLA Medical Center between March 1984 and February 1990 were analyzed. Overall, 17 patients were found to have HCV viremia by RT-PCR assay, of whom 14 had a positive second-generation HCV enzyme immunoassay (EIA-2) and only 6 were EIA-1 reactive. The frequency of HCV viremia increased with the duration of time between diagnosis and sample procurement, and the number of blood products transfused prior to sampling (*P* = 0.026). No patient who received fewer than 20 U of blood products or who was sampled less than 20 days after diagnosis had a positive HCV RT-PCR result. Of four patients with hepatitis-associated AA (HAAA), one who was sampled 23 days after diagnosis had hepatitis C viremia and a reactive EIA-2 assay. Therefore, the high frequency of HCV viremia in this patient population is most likely due to transfusion with contaminated blood products prior to the introduction of routine blood donor screening for HCV. Am. J. Hematol. 58:122–126, 1998.

Key words: aplastic anemia; hepatitis C; transfusion; RT-PCR

INTRODUCTION

Aplastic anemia (AA) is characterized by peripheral blood pancytopenia and bone marrow hypocellularity in the absence of an infiltrative process. The etiology of AA in the majority of cases is unknown but exposure to various drugs, benzene, or ionizing radiation can be causally implicated in some cases. Approximately 1% of AA patients present within 6 months of a clinically recognized episode of hepatitis [1]. Case reports suggest that hepatitis A or B may infrequently cause bone marrow aplasia. However, most hepatitis-associated AA (HAAA) is not related to infection by these agents [1–4].

Hepatitis C virus (HCV) is a single-stranded RNA virus similar to flaviviruses [5]. The best documented mode of virus transmission is parenteral, which occurs most commonly during blood transfusion or sharing of needles by intravenous drug users; the role of sexual or vertical transmission appears to be minimal [6–8]. The HCV causes an acute hepatitis that is most commonly mild or asymptomatic [9]. Despite the relatively benign nature of the primary infection, approximately 70% of infected individuals develop chronic hepatitis [6]. The incidences of both HCV infection and AA are higher in Southeast Asia than in Western Europe and North America, suggesting a possible association between these

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disorders. The often occult nature of primary and chronic HCV infections suggests that an association between AA and hepatitis C could be under-appreciated.

The detection of HCV by serologic methods can be confounded by the protracted time, up to several months, required for seroconversion in some patients. Immunosuppression, such as that administered to AA patients, might delay or abort seroconversion to some HCV antigens. Therefore, a method to directly detect HCV, such as the reverse transcriptase polymerase chain reaction (RT-PCR) for HCV RNA, could be a much more sensitive indicator of infection in AA patients. We analyzed the sera from a large number of AA patients using multiple serologic tests and RT-PCR to ascertain the frequency of HCV infection in this population.

MATERIALS AND METHODS

Patients

Serum samples were collected from 90 AA patients who presented to UCLA between March 1984 and February 1990, and who consented to enrollment in IRB-approved treatment protocols. Samples were obtained a median of 51 (range 2–1,759) days after diagnosis. The patients had received a median of 34 (range 0–638) prior blood product transfusions. The serum samples were frozen at −70°C until they were analyzed. Clinical information, laboratory results, and transfusion histories were obtained from hospital charts or computerized files. Approval for collection of clinical data was obtained from the IRB.

HCV Serologies

Sera from AA patients were analyzed for the presence of anti-HCV antibodies using the enzyme immunoassays EIA-1 and EIA-2 (Ortho Diagnostic Systems, Raritan, NJ), and the HCV RIBA-2 strip immunoassay (SIA; Chiron Diagnostic, Emeryville, CA). All serologic assays were performed according to the instructions of the manufacturer. The HCV EIA-1 was positive if the result was greater than the negative control mean plus 0.4; the HCV EIA-2 was positive if the result was greater than the negative control mean plus 0.6. The HCV RIBA-2 SIA was performed only on samples that were positive by EIA-1. The RIBA-2 assay was negative if no bands had ≥1+ or the hSOD band alone was reactive, and was positive if at least two bands corresponding to antigens encoded by different parts of the HCV genome were ≥1+. An indeterminate interpretation was rendered if neither positive nor negative criteria were satisfied.

HCV Detection

Serum samples were assayed for HCV RNA using the AMPLICOR Hepatitis C Virus Test (Roche Diagnostic Systems, Branchburg, NJ). Briefly, HCV RNA was reverse transcribed using a biotinylated primer downstream of the 5′-untranslated region of the HCV genome, *Thermus thermophilus* DNA polymerase, and deoxynucleotide triphosphates. PCR then was conducted using an unbiotinylated upstream primer for forty cycles. The PCR products were denatured and hybridized with an oligonucleotide probe to the HCV 5′-untranslated region in microwell plates. Bound PCR product was incubated first with avidin-horseradish peroxidase, then peroxide and tetramethylbenzidine, resulting in a colored complex. The microtiter plates were read at 450 nm using a Biotek 312e EIA Reader. Results were interpreted according to the manufacturer's instructions.

TABLE I. HCV RT-PCR and EIA Results in AA Patients*

	% Positive	
HCV RT-PCR	EIA-1	EIA-2
Positive (17)	35	82
Indeterminate (8)	37	37
Negative (65)	3	12

*Numbers in parentheses are numbers of patients. HCV, hepatitis C virus; RT-PCR, reverse transcriptase polymerase chain reaction; EIA, enzyme immunoassay; AA, aplastic anemia.

Hepatitis B Testing

All serum samples were evaluated for the presence of the hepatitis B surface antigen (HBsAg) using a standard EIA technique.

Hepatitis E Testing

An EIA for antibodies to the hepatitis E virus (Genelabs, Inc., Redwood City, CA) was performed according to the instructions of the manufacturer. The assay was interpreted as positive if the result exceeded the negative control mean plus 0.5.

RESULTS

HCV RT-PCR

A total of 17 patients had detectable HCV viremia at the time of presentation to UCLA, and an additional 8 patients had indeterminate RT-PCR results (Table I). Patients with HCV viremia had received a median of 113 (range 22–392) blood product transfusions prior to providing a serum sample, which was obtained a median of 136 days (range 23 to 881 days) after diagnosis. Positive results were more often observed in patients who had received a higher number of transfusions, and were seen only in patients who had received >20 blood product transfusions prior to sampling (Fig. 1). A positive RT-PCR result was also more common if the time from diagnosis to sampling was >40 days (Fig. 2). Patients with HCV infection had significantly more prior trans-

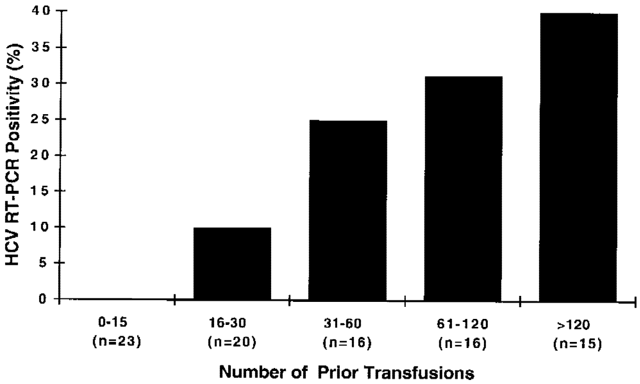

Fig. 1. The frequency of HCV infection increased with higher numbers of blood product transfusions received by AA patients.

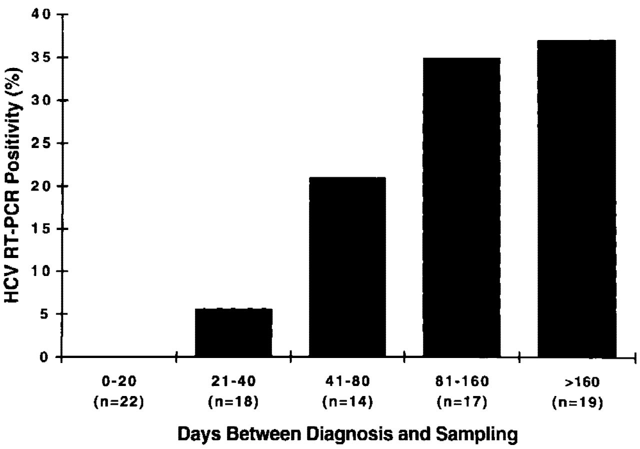

Fig. 2. The frequency of HCV infection increased with time from the diagnosis of AA.

fusions than patients without viremia ($P = 0.026$; two-tailed t-test).

HCV Serologies

Eleven of 90 samples from AA patients tested positive for HCV antibodies by EIA-1 and 25 were reactive by EIA-2. All of the EIA-1 positive samples were reactive using EIA-2, but only 27% were RIBA-2 positive. Similarly, 27% of sera positive by EIA-2 were RIBA-2 reactive. Of the 17 patient samples that were RT-PCR positive, 14 (82%) were EIA-2 reactive, but only 6 (35%) were EIA-1 positive (Table I). Three of 8 (38%) RT-PCR-indeterminate samples were reactive by both EIA-1 and EIA-2. Samples negative for HCV viremia by RT-PCR were sero-positive in 3% or 11% of assays using EIA-1 or EIA-2, respectively.

Hepatitis-Associated Aplastic Anemia

Four patients had hepatitis that preceded or coincided with the diagnosis of AA (Table II). One patient with evidence of hepatitis at the time of AA diagnosis had HCV viremia, a positive EIA-2 result, but a negative RIBA-2 assay. The serum sample was obtained 23 days after diagnosis, by which time the patient had been transfused with 22 U of blood products. An additional patient had resolving hepatitis at presentation, was HCV RT-PCR negative, HCV EIA-1 and EIA-2 positive, but RIBA-2 negative. This patient had received 30 U of blood products within the 13 days prior to obtaining the serum sample. Two other patients had clinical hepatitis approximately 8 months prior to presenting with AA, by which time their liver function tests had normalized. Neither patient had detectable HCV by RT-PCR, or a positive HCV EIA (Table II).

Hepatitis B or E Infection

Five patients had a positive EIA for HBsAg but no recent history of hepatitis. These patients had a median of 37 (range 17–392) antecedent blood product transfusions and were diagnosed a median of 52 (range 23–881) days prior to sampling. Two patients positive for HBsAg also had HCV viremia. Three patients had positive hepatitis E serologies but none had a history of clinical hepatitis.

DISCUSSION

We identified a high frequency of HCV viremia, with concomitant seropositivity by EIA-2 assay, in this group of 90 AA patients. This suggests that the majority of viremic patients were infected at least several weeks prior to sampling. Studies of transfusion-associated hepatitis C have demonstrated an interval of 10–39 weeks between the time of HCV infection and seroconversion using first-generation immunoassays, while second-generation assays may become positive as early as 6 weeks after HCV exposure [9–11]. HCV RNA can be detected within 1 week of transfusion of infected blood products, and by 2 weeks virtually all recipients of an infected blood product have detectable RNA. The median time from AA diagnosis to serum sampling was 136 days (range 23 to 881 days) for patients with a positive HCV RT-PCR test and 106 days (range 11 to 1,492 days) for those with a positive EIA-2 assay. Therefore, there was adequate time for most patients with either a positive RT-PCR assay or a reactive EIA-2 test to have acquired HCV infection from blood product transfusion.

The EIA-2 test was much more sensitive in detecting HCV infection than was the EIA-1 assay in our patient population. The EIA-2 test was reactive for 82% of RT-PCR positive samples while the EIA-1 assay identified only 38% of the viremic patients. The RIBA-2 assay was also insensitive; only 28% of RT-PCR positive samples tested were RIBA-2 reactive. Because seroconversion by EIA-2 can be more rapid than by EIA-1, the differences in the sensitivities of these two assays could reflect a high

TABLE II. Patients With Hepatitis-Associated Aplastic Anemia*

Patient	HCV PCR	HCV serologies			HBsAg	Day of sample[a]	Total previous transfusions[b]	Time from hepatitis to aplastic anemia
		EIA-1	EIA-2	RIBA-2				
1	+	+	+	−	−	d+23	22	Concurrent
2	−	+	+	−	−	d+13	30	1 month
3	−	−	−	ND	−	d+8	30	8 months
4	−	−	−	ND	−	d+36	41	8 months

*HCV, hepatitis C virus; PCR, polymerase chain reaction; EIA, enzyme immunoassay; HBsAg, hepatitis B surface antigen; ND, not determined.
[a]Day following diagnosis.
[b]Units of blood products transfused.

proportion of relatively recently infected individuals in our patient population.

The high rate of seroconversion among HCV RT-PCR positive patients suggests that humoral immunity may be normal in AA. A number of immunologic abnormalities have been observed in AA patients including decreased numbers of circulating B lymphocytes, increased numbers of activated suppressor/cytotoxic T lymphocytes in the blood, defective macrophage differentiation, and decreased circulating levels of interleukin-1 [12–17]. In addition, AA patients often receive immunosuppressive medications as part of their treatment; many of our patients were treated with glucocorticoids prior to their referral. Therefore, the high prevalence of anti-HCV antibodies in our AA patients with HCV viremia suggests that they do not have a defect in humoral immunity.

The incidence of HCV viremia in the AA patients increased with the number of prior transfusions and the length of time between diagnosis and sampling. This suggests a direct relationship between blood product transfusion and HCV infection. A number of studies have addressed the frequency of HCV infection in volunteer blood donors during the time that the AA patients were receiving transfusions. Random blood donors in New York had a 0.9% frequency of anti-HCV antibodies by radioimmunoassay during 1985–1986 [18]. However, because no confirmatory assay was performed, it is likely that this frequency is an overestimation. A multicenter U.S. study evaluated the rate of HCV seroconversion rate among 2,410 patients who were transfused because of cardiac surgery [19]. Patients were considered to be seropositive for HCV if they had reproducibly positive ELISA tests and a positive RIBA assay. The rate of seroconversion was 0.45% per unit transfused between 4/85 and 9/86, and 0.19% per unit given between 10/86 and 5/90. Another multi-institutional U.S. study investigated the incidence of HCV seropositivity among 14,068 individuals who donated blood in 3/91 [20]. Using the EIA-2 assay, 0.5% of the samples were repeatedly positive for HCV antibodies. Of the EIA-positive samples, 39% were RIBA-positive and 30% were indeterminate. PCR analysis found that 89% of samples positive for both EIA and RIBA had detectable HCV RNA, and 19% of EIA-positive, RIBA-indeterminate samples were PCR-positive. The overall frequency of donors with HCV viremia was approximately 0.2%. The frequency of HCV infection in our AA patients (0.32%/U) was not unexpectedly high given the prevalence of HCV infected blood products at the time that they were being transfused.

Several studies have addressed the potential involvement of HCV infection in the pathogenesis of acquired AA. A French study found that 10% of 118 patients with severe AA had anti-HCV antibodies using an ELISA assay [21]. The frequency of seropositivity was higher in patients with hepatitis-associated AA (16%) than in those with AA due to other causes (9%), but not significantly so. The patients with HAAA were subsequently found to have frequencies of RIBA-2 and HCV RT-PCR positivity that were similar to those of matched controls with AA due to other causes [22]. An international study found a relatively high frequency (36%) of HCV viremia by PCR in 28 patients with hepatitis-associated AA [23]. However, the presence of HCV viremia correlated with the number of blood transfusions obtained prior to sampling. A recent study of 10 HAAA patients found all of them to be negative for both anti-HCV antibodies and HCV RNA [24]. A series of 53 previously untransfused AA patients from Thailand had a 6% frequency of HCV viremia, which was similar to that of controls [25]. Nevertheless, there is at least one case report of a patient developing AA after a well-documented infection with hepatitis C [26]. One of 4 patients in our series with HAAA had HCV viremia and positive EIA-1 and EIA-2 assays. This patient had received prior blood products but was sampled only 23 days after initial transfusion, suggesting that there was inadequate time for seroconversion if the blood products were a source of HCV infection.

Therefore, a high rate of HCV viremia, detected by RT-PCR, and HCV seropositivity using the EIA-2 assay were observed in this AA patient population. The high frequency of HCV infection can adequately be explained by the prevalence of HCV-infected blood products in use at the time these patients were being transfused. Our

results suggest that HCV infection is not a frequent etiology of AA, but a role for HCV in occasional cases of HAAA cannot be entirely excluded.

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